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T-DNA Mediated Activation Tagging in Arabidopsis: A Powerful Tool for Gene Discovery

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Abstract—*Rapid improvements in the sequencing technologies have led to the availability of genome sequences of severalorganisms. The recent advances made in bioinformatics tools have played a major role in predicting the gene models as well asin identifying the protein-coding transcripts. Various reverse and forward genetics strategies have been followed to determine the functions of these gene models and regulatory sequences. A significant limitation of classical loss-of-function screens designed to dissect genetic pathways is that they rarely uncover genes that function redundantly, are compensated by alternative metabolic or regulatory circuits, or which have an additional role in early embryo or gametophyte development. Activation tagging is one approach that has emerged in plants to help circumvent these potential problems. Using T-DNA or transposons as tags, significant progress has been made by using "Knock-in" approaches ("gain-of-function" or "activation tagging") in different plant species. This technique utilizes a T-DNA boarder sequence that contains four tandem copies of the cauliflower mosaic virus (CaMV) 35S enhancer sequence. The element enhances the expression of neighboring genes either side of the randomly integrated T-DNA tag led to the ectopic expression of the nearby gene, resulting in gain-of-function phenotypes. Through activation tagging a number of genes has been identified which are fundamental to development, metabolism and disease resistance in Arabidopsis. Here we discuss the genes isolated and cloned in our lab through activation tagging by observing the novel phenotype governed by the respective gene. We also discuss the construction of new more sophisticated vectors for the generation of conditional alleles which significantly accelerate the process of gain-of-function genetics in plants.*